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NUTRITION REVIEWS

VOL. 19 MARCH 1961 No. 3

WHY IS SCIENCE IMPORTANT?*

It may seem queer, trivial, and even fatuous to ask why science is important. Is not every person ready to concede the answer? Are we not surrounded almost every minute of our lives by evidence that science is both powerful and successful?

During the recent political campaign we heard rumors that the prestige of the United States had decreased, but we have heard no such rumors about science!

A few people might deplore certain of the accomplishments of science. A few might so misunderstand science as to view it as a mechanical and unpleasant enemy of what they think of as the softer, lovelier, more poetic aspects of the good life. A few might wish to continue the older misunderstandings between science and religion and falsely claim that science is a foe of faith. There have even been a few who, seeking a victory by retreat, have suggested a moratorium on science.

But by and large, science is conceded to be in the front row, and it is as questionable to challenge science as it would be to scoff at Mother's Day. Why, then, the question "Why Is Science Important?"

Science is often misrepresented, and as a result it gets a great deal of respect (including some awe and fear) which it does not deserve; and contrariwise, it gets far too little of the credit that it does in fact deserve. Four reasons why science is important are presented, in ascending order of significance, with clear implications of why all citizens of our free democracy must know more about science—about *real* science—than they do at the present time.

The most obvious reason why science is important is that it creates all the basic knowledge which, when applied, leads to a myriad of procedures and things that make life healthy, comfortable, and pleasant. It is this union of basic knowledge and practical application which enables medicine to cure us when we are ill, which enables the food producers and processors to feed us when we are hungry, which makes more effective our ever-increasing periods of health, and which extends the span of our living.

The advances in medicine and public health have been phenomenal. From 1944 to 1957 the United States death rate from diphtheria was decreased by 96%. For scarlet fever and whooping cough, the decrease in each case was 90%, while for tuberculosis the decrease was 80%. Since 1930 the average life span of our citizens has been increased by about ten years. When you go into a drugstore today, the chances are nearly even that if you carry a prescription it is one that could not have been filled five years ago. About 75% of present-day prescriptions would not have been fillable 25 years ago because the necessary chemicals, drugs, and antibiotics have been developed since then. This is the sort of progress which is possible when the basic sciences of organic and biochemistry, of physiology, and of pharmacology team up with (and within) the great pharmaceutical industries.

Science produces the basic knowledge of the laws of nature which enables technology to clothe us with new materials of superior

* Adapted from a talk before the Board of Trustees of the Nutrition Foundation on October 27, 1960, a modified version of which appeared in Chemical & Engineering News, February 13, 1961.

qualities and to shelter us in buildings of efficient beauty with wholly specified interior climates and with shadowless lighting. This union of science and technology heats us when we are cold and cools us when we are hot; transports us with almost unbelievable speed and comfort (with the exception of commuting travel, where the breakdown seems to center in the social rather than in natural science); furnishes us with a wide variety of ways—telephone, radio, and television—in which we can communicate, these being so efficient that the claim each day over the radio, "News with the speed of sound," is too modest by a factor of almost exactly one million (which certainly must be the Madison Avenue record for understatement).

This union of science and technology puts in our storage pantries, our ice boxes and freezers, and on our tables, an array of foodstuffs which, in attractiveness, taste, and nutritional value, exceeds anything the world has previously known.

The technologies made possible by science are, in the near future of great expansion and improvement of our educational processes, surely going to render essential new types of service, as is illustrated by the television programs which will presently be broadcast from an airplane circling high over north-central Indiana, six hours of every school day. These programs will be available to more than five million students in more than 13,000 elementary schools, high schools, colleges, and universities in parts of six states; and eventually six simultaneous taped programs can be transmitted from this single source.

Technological achievements furnish our homes with every conceivable device for increasing our comfort and for reducing our labor. We do not have to walk across the room to tune our television sets; and presently there will be available electronic devices by which one can brighten or dim the lights, open or close the windows, and rapidly cook a meal, all without getting out of bed.

The productive marriage of basic science and technology, which has illustrated the first reason for considering science important, also illustrates the claim that science is given too much credit for wrong reasons and too little credit for right reasons.

For far too many persons think that science in fact *consists* of developing electric can openers, or bigger rockets, or longer-lasting house paints, or more rapid dialing systems for long-distance telephoning, or wrinkle-proof clothes, or better air conditioning systems.

However, all these desirable adjuncts of modern living, although in many instances they are made *possible* by science, certainly do not *constitute* science. Although science deserves considerable credit for all these useful and attractive devices, materials, and procedures, in the sense that basic scientific knowledge usually (but not always) is a prerequisite to such developments, technology deserves the credit for having the imagination, the financial courage, the ingenuity, and the driving energy to see to it that so-called "pure knowledge" is in fact brought to the practical service of man. And it should be most strongly emphasized that those who have the urge to apply knowledge usefully have themselves often made significant contribution to pure knowledge and have even more often served as a stimulation to the activities of the pure researcher.

All of this is old hat to distinguished scientists and industrial leaders. But *all citizens* must be educated to recognize the true nature of the symbiotic relationship between science and technology and the extent to which each depends upon the other. They must realize that the advance of technology depends essentially upon the continuing flow of basic new knowledge concerning nature, that the responsibility for that flow rests primarily upon those who devote their lives to basic research, and that the deepest, most original, and apparently the most *impractical* theoretical discoveries of the pure scientist eventually,

through technology and engineering, result in the practical devices, materials, and procedures which make life safe, comfortable, efficient, and pleasant.

Especially must the general public be brought to realize that it is wrong to be impatient with basic science, that it is self-defeating to put too much emphasis upon practicality and upon prompt returns from pure research. It is most gratifying that there have been recent evidences of recognition, even at the highest political levels, of an understanding of this point. Mr. Nixon, in a statement made during the campaign, said, "Because of our heritage of expecting practical achievement from effort we too often inquire of the basic research scientist, 'What discovery do you plan to make?' or 'What can be done with it?' We must realize that this type of impatience for immediate results can be catastrophic. It prevents our basic research scientist from exercising his true function—achieving knowledge for its own sake."

And President Theodore Heuss of West Germany, with deep understanding, has said, "We have the obligation to free the scientist from the nervousness of this era and to put the gift of time again at his disposal."

Yes, the practical fruits of science constitute an impressive reason for considering science important. The second claim for science is very closely connected with the first one. Science, in fact, is important because its health and its vigorous growth are essential to the economic health and growth of our country.

This point is so obvious and so generally recognized that one does not need to belabor it. A very tangible confirmation is furnished by the record of stock prices on the Exchange. We have all seen what happens to the Big Board quotations of companies, such as IBM, Polaroid, and Beckman, which are recognized as maintaining a strong and competent emphasis on research in new and expanding fields. When General Telephone becomes General Telephone *and* Electronics, there is a substantial market reaction. When a company previously noted primarily for manufacturing toy trains decides to branch out into activities related to the almost-magic terms control engineering, automation, and electronic devices, the reaction is prompt and impressive.

Indeed one could be a little cynical over the fact that in our newspapers basic research sometimes seems to be respected and praised in the financial sections rather more than it is in the general news section or in the editorials. There can be no doubt: the very ponderable argument of the balance sheet of industry strongly testifies that science is important.

The third reason for considering science important is, again, closely related to what has already been said. And again it is true that the argument is so clear and so powerful that detailed substantiation is unnecessary. It is obvious that science plays a central role in the great and essential task of defending our country, and in the still broader task of defending at various places in the Free World the philosophy which states that what is important is the union of collective responsibility with individual freedom.

Some of us remember World War I, which has sometimes been referred to as "the chemists' war," because of the development at that time of offensive and defensive measures of chemical warfare, and sometimes as "the physicists' war," largely because of the importance of the role which physics played in submarine detection. The first primitive gyroscopic devices for indicating turn and bank in an aircraft were made at that time. When one thinks of the exquisite refinement of present-day gyroscopic devices used in inertial guidance and in many other applications, he realizes how much progress has been made. The advanced specialists in servo control theory, in telemetering devices, in plasma physics, in cryogenics, in the production of new materials with great resistance to heat, in solid

state physics—indeed in the most abstruse mathematical aspects of game theory—these scientists are, to a very important and remarkable degree, the modern defenders of democracy.

It must, moreover, be recognized that the modern defenders of democracy are by no means limited to the military field. The prestige of our country and the world-wide respect for our ideals depend to a significant degree upon our scientific effectiveness in solving planetary problems of health and agriculture; upon the success which we have in certain spectacular areas such as high-energy physics, space research, radio astronomy, arctic research; and, more broadly and fundamentally, upon the general richness, vigor, and imaginative character of our national scientific activity. Not for the lesser reason of competitive success, but for the higher motivation of responding worthily to our opportunities, we must see to it that our entire national effort in science is second to no other.

The fourth and final reason why science is important is the most outstanding one. For thousands of years during his early development man lived in a terrifying world of mystery and confusion, with darkness, storm, wind, and wave, all evil enemies, with an eclipse a portent of doom, with chaotic and irrational disorder constantly threatening.

The development of the reasoning mind, of the experiment that seeks the facts, of the combination of analytical and poetic power which constructs hypotheses which may be tested, of the surging faith that the universe is reasonable and orderly and the discovery that it is lovely and beautiful almost beyond belief—these are the real reasons why science is important.

Einstein once remarked, with an artistic quality of contradiction which was characteristic of his mind, so humble and simple but so special and profound, that the most incomprehensible thing about the universe is that it is comprehensible. Not by any means has all of the incomprehensibility of the universe been clarified by science. The creative arts and religion—especially the latter—are responsible for illuminating and enriching vast fields of experience inaccessible to science.

The capacity of science to reveal the order and beauty of the universe—from the most evanescent elementary particle up through the atom, the molecule, the cell, man, our earth with all its teeming life, the solar system, the metagalaxy, and the vastness of the universe itself—constitutes the real reason, the incontrovertible reason, why science is important.

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AMINO ACID EXCRETION IN INFANTS AND CHILDREN

Generally speaking, young infants excrete larger quantities of amino acids than do older children. At all ages the variability among subjects is wide.

The development of suitable microbiological and chromatographic techniques in recent years has stimulated the study of amino acid metabolism in man. With this development has come the realization that disordered amino acid metabolism may be associated with a number of clinical syndromes, the prototype being phenylpyruvic oligophrenia. Most of these syndromes have been considered to be due to enzymatic malfunction and are thus classed under the general term "inborn errors of metabolism". Most of them become manifest during infancy and childhood. Currently, efforts are being made to discover additional

abnormalities in patients suffering from a variety of disease states.

Assay of the urine for amino acid content is the first step in the investigation of patients suspected of harboring such an abnormality. For comparison, information must be available on the excretion pattern in normal individuals. Previous reviews on this matter (*Nutrition Reviews* **6,** *6 (1948)*; **7,** *68 (1949)*) are concerned solely with the adult, although total alpha-amino nitrogen excretion was reported for 16 normal children in 1955 (*Ibid.*, **13,** *266 (1955)*).

It is of particular interest to review the findings in three recent articles devoted to a study of excretion patterns of individual amino acids in children and infants, including the prematurely born. H. Ghadimi and H. Shwachman (*Am. J. Dis. Child.* **99,** *457 (1960)*) present data on total alpha-amino nitrogen and creatinine in 35 healthy infants and children and in 65 additional infants and children suffering from a variety of diseases. D. O'Brien *et al.* (*Acta Paediat.* **49,** *258 (1960)*) give data on 11 healthy premature infants with regard to excretion of 15 amino acids and glutamine. Creatinine, total nitrogen, and total amino nitrogen were also measured. H. K. Berry (*Pediatrics* **25,** *983 (1960)*) studied some 220 normal subjects aged four weeks to 10 years. Seventeen amino acids were measured, as well as creatinine, glutamine, beta-aminoisobutyric acid, and an unidentified ninhydrin "spot". Paper chromatographic techniques were used for the assay of the individual amino acids.

The amino acids assayed in Berry's series were serine, glycine, threonine, alanine, glutamic and aspartic acids, lysine, tyrosine, valine, taurine, histidine, proline, hydroxyproline, phenylalanine, cystine, arginine, and leucine-isoleucine. O'Brien *et al.* give values for all of these except phenylalanine and arginine. These data may be compared to those of other workers, some of whom used column chromatography or microbiological methods. The latter refer to the work of K. Schreier and H. Pluckthun (*Biochem. Z.* **320,** *447 (1950)*); T. H. J. Huisman (*Arch. franç. pediat.* **14,** *1 (1957)*); D. Fowler *et al.* (*Arch. Biochem.* **68,** *452 (1957)*); and L. I. Woolf and A. P. Norman (*J. Pediat.* **50,** *271 (1957)*); also articles by J. P. Dustin and H. Bickel (*Die Physiologische Entwicklung des Kindes, ed. by F. Linneweh, Berlin, Springer-Verlag (1959)*).

In premature infants total amino nitrogen rises from an average value of 5 mg. per kg. of body weight per day during the first week of life to 14–16 mg. by the fourth week, there being no further change through the eighth week. Expressed as percentage of total nitrogen in the urine there is little change (range 4–9 per cent) throughout this period. Values for later infancy and childhood are lower—2 mg. of amino nitrogen per kg. of body weight daily (0.7 per cent of total nitrogen). There is considerable variation among individuals, with coefficients of variation ranging up to 50 per cent and beyond.

The range of variation in the excretion of the various individual amino acids was also wide. However, the trends with increasing age are definite. Glycine excretion, for example, is about 450 μg. per mg. creatinine in the first month of life, 170 μg. at one year of age, and 35 μg. at 9–10 years. For each of the eleven amino acids measured by Berry over a wide age range, excretion was higher in the first year of life than later in childhood. Proline and hydroxyproline were detected in the urine of all of the premature infants by O'Brien *et al.*; Berry found that, relative to creatinine, the excretion of each dropped markedly during the first seven months of life.

For instance, the degree of variation among subjects is exemplified by the fact that lysine was excreted in relatively large amounts by some infants and could not be detected in others. Berry attempted to relate some of the variation to diet. In general, infants fed cow's milk excreted greater quantities of valine, leucine, lysine, tyrosine,

glutamic acid, and threonine than those fed human milk, but the differences were not great. Repeated determinations in one infant during the first 21 weeks of life revealed a constant pattern (except for age changes) in the face of a gradual change in diet. Furthermore, clinical data were obtained on 11 infants and children who showed a marked increase in the excretion of one or more amino acids. Some of these had definite organic diseases such as asthma, infection, or cardiac decompensation; others appeared normal in every respect. Five pairs of twins were studied: concordant excretions of lysine, threonine and tyrosine were found.

These studies, as well as those by other workers, provide valuable information on the pattern of amino acid excretion in the infant and child, information needed in order to properly evaluate such patterns in various disease states.

The reasons for the general tendency for excretion rates to decline with advancing age of the child were considered only briefly by the authors. The fact that protein intake on a per kilogram basis drops with age is one possibility. Another is the well established fact that renal tubular reabsorption mechanisms are not well developed in the young infant.

Even a brief survey of the extant literature in this field is enough to convince one of the need for uniformity in the manner of presentation of the data. Some authors refer everything to body weight, others to creatinine excretion. The use of both micromols and micrograms is also confusing.

EFFECTS OF ANIMAL VERSUS VEGETABLE PROTEIN ON SERUM LIPIDS OF MAN

Healthy young women were fed diets containing either 50 g. of vegetable protein or 50 g. of animal protein. Measurements of serum cholesterol concentration disclosed a significant decrease in both groups, with a greater decrease in those fed the vegetable diet; study design did not indicate which factor or factors might have caused the decrease.

The importance of factors which regulate the amount of cholesterol in the serum of healthy persons is readily apparent. There are abundant reports to indicate that the total intake of calories, the relative amount of fat in the diet, the type of fat and the amount of animal protein all are significant in determining the concentration of the cholesterol in the serum. Recent evidence indicates that dietary cholesterol also exerts a significant effect upon the amount in the blood (*Nutrition Reviews* **18,** *328 (1960)*).

M. G. Hardinge and F. J. Stare (*J. Clin. Nutrition* **2,** *83 (1954)*) reported that true vegetarians had remarkably low concentrations of cholesterol in their serum, that vegetarians who ate eggs and milk products had a somewhat higher level, and that non-vegetarians had the highest level. Later, R. E. Olson *et al.* (*Am. J. Clin. Nutrition* **6,** *310 (1958)*) reported that the concentration of cholesterol in the serum could be reduced significantly by decreasing the amount of dietary protein of healthy individuals. To evaluate the significance of small amounts of protein in the diet, or of the source of protein (animal versus vegetable), G. R. Walker, E. H. Morse, and V. A. Overley (*J. Nutrition* **72,** *317 (1960)*) fed special diets to 12 healthy young women. Six of the subjects were given a diet which contained primarily vegetable proteins and the other six were fed a diet containing animal proteins. The total amount of protein in each diet was low: approximately 50 g. per day or 8 per cent of the calories. Actually, the subjects eating a vegetable protein diet received some animal protein in the form of whole milk and coffee cream. The fat content of both diets was approximately 95 g. per

day or 36 per cent of the calories. This was supplied in the form of hydrogenated soybean and cotton seed oils, other hydrogenated vegetable shortenings, dairy fats, and salad oil. Although the group fed an animal protein diet received a small additional amount of fat in their meat, the degree of saturation of the fats in the two diets was quite similar. The average caloric intake was 2370 per day.

The subjects ate these diets for six weeks. Blood was drawn at the end of each week for determination of concentrations of cholesterol, serum lipid phosphorus, and serum fatty esters. The concentration of cholesterol in the serum of both groups decreased significantly from its initial values. However, there was a significantly greater decrease in the cholesterol of the vegetable protein group at the end of the second and fifth weeks. There also was some decrease in the concentration of lipid phosphorus in the sera of the vegetable protein group, but there were no differences in the serum fatty ester concentrations. The authors considered that the reduction in concentration of serum cholesterol might have been due to the large amount of vegetable fats in the diet which provided relatively large quantities of unsaturated fatty acids, essential fatty acids, and sitosterol.

Although this study was carefully conducted and did show significant changes in lipid metabolism, it was not designed so that it would yield specific information regarding which factor or factors might have caused the decrease in the cholesterol. For example, the reviewer estimated that the vegetable protein diet contained 30 mg. of cholesterol as compared with 80 mg. in the animal protein diet. According to J. M. R. Beveridge (*J. Nutrition* **71,** *61 (1960)*) this difference alone might have been adequate explanation for the changes observed. In addition, the simultaneous changes in quantity of protein, in relative amount of fat, in iodine number of the fats, and in the amount of sitosterol contained therein as compared with the normal diet of the subjects, introduced too many variables to allow definitive evaluation. This study does yield useful data, but the results might have been a consequence of the change in the amount of protein or the change in the type and quantity of fat or the reduction in dietary cholesterol.

I^{131} FAT-PANCREATIN TEST FOR STEATORRHEA

Pancreatic extract increases the absorption of I^{131}-triolein in patients with pancreatic insufficiency, but not in those with "malabsorption" syndromes. The test is of diagnostic value.

The use of I^{131} as a radioactive label has simplified the study of fat absorption (*Nutrition Reviews* **15,** *171 (1957)*; **17,** *10 (1959)*) and made it easier to distinguish between patients with impaired fat digestion (*e.g.* lack of pancreatic lipase) and those with impaired fatty acid absorption (various malabsorption syndromes). I^{131}-labeled fat and fatty acid have been used for the latter purpose (*Ibid.* **17,** *211 (1959)*).

Using a somewhat different approach, A. A. Polachek and R. F. Williard have observed the effect of pancreatic extract on the absorption of I^{131}-labeled fat as a diagnostic test in patients with steatorrhea (*Ann. Int. Med.* **52,** *1195 (1960)*). They studied seven patients with pancreatic insufficiency and seven with malabsorption syndrome. The former all had calcific pancreatitis; six of them also had diabetes mellitus. The group with malabsorption syndrome consisted of two patients with sprue, two with diverticulosis of the whole intestinal tract, one with avitaminosis and tabes dorsalis, one with lymphosarcoma of

the small intestine, and one with severe passive congestion of the small intestine.

Preliminary administration of potassium iodide served to block thyroid uptake of the test I^{131}. The fasting subjects were given an emulsion of I^{131}-triolein in peanut oil, and plasma radioactivity was determined at suitable hourly intervals. The test was repeated on a later day with the addition of 3 g. of a hog pancreatic extract of high lipolytic activity.

The mean I^{131} plasma levels of the patients with calcific pancreatitis were much higher when pancreatic extract was given than without it (9.8 versus 3.4 per cent at the peak of the curves, about five hours after the test meal). Individual cases showed clear differences between the peak plasma I^{131} levels with and without pancreatic extract (from 35 to several hundred per cent). Patients with malabsorption syndromes, as expected, showed very little effect of the extract. (The mean plasma I^{131} levels were 6.4 versus 6.0 per cent).

This test distinguishes the two groups of patients rather clearly. Only one patient in each group is not distinguished by the procedure (the patient with the least difference in the pancreatitis group and the one with the greatest difference in the malabsorption group; the latter had sprue). Most of the patients with malabsorption had higher I^{131} plasma levels (without pancreatic extract) than those with pancreatitis.

Although the test appears simple and clinically useful, more information would be helpful in appraising it. The authors do not give any clinical data except for the diagnoses. Specifically, the steatorrhea is not documented; one infers from the title of the article that the patients all manifested it. The group with malabsorption syndrome is heterogeneous. Five different diagnoses are included; for all except sprue, the reader must accept the diagnosis of malabsorption syndrome on faith. At least two of these patients (the one with avitaminosis and the one with passive congestion) absorbed triolein better than two of the pancreatitis patients did after pancreatin. Normal controls are not given, but one infers that these two patients with malabsorption did not have severe defects of fat absorption.

MALABSORPTIVE SYNDROME INDUCED BY NEOMYCIN

Administration of a poorly absorbed antibiotic which reduces the intestinal flora was found to induce histological changes in the mucosa similar to those noted in sprue.

Steatorrhea, azotorrhea and interference with the absorption of carotene, glucose, radioactive iodine, and vitamin B_{12} have been found to occur in individuals taking orally 12 g. of neomycin sulfate a day. This antibiotic is used extensively in reducing the bacterial flora of the intestinal tract before surgery.

In studying this malabsorption syndrome, E. D. Jacobson, J. T. Prior and W. W. Faloon (*J. Lab. Clin. Med.* **56,** *245 (1960)*) examined the jejunal mucosa of 14 subjects who had been receiving the antibiotic. Eleven of the individuals were chosen because they were to undergo abdominal surgery in which the jejunal surfaces would be exposed and biopsy of the mucosa could be obtained readily. Five patients receiving the usual preoperative therapy and diet were used as controls for the group receiving neomycin. Six patients were given neomycin sulfate, 4 g. three times a day for a period ranging from four to 33 days prior to the operation. In the three remaining patients jejunal mucosal biopsies were obtained using the Crosby peroral jejunal biopsy capsule. This instrument makes an operative procedure unneccessary. In one patient individual biopsy was obtained before he received the neomycin, again after eight

days of treatment, and finally 18 days after the neomycin had been withdrawn. In the other two subjects biopsies were obtained on the fifth to tenth day while they were receiving the antibiotic.

Biopsies were obtained from the proximal jejunum. The changes obtained on histological examination varied in magnitude, but mucosal samples from all subjects treated with neomycin showed the characteristic patterns. Such changes were not found in the untreated subjects. Following neomycin therapy, the mucosal villi showed edema and increased cellular infiltration and changed their form becoming polypoid, plateaued, or sometimes mushroom shaped. All the villi did not undergo changes since there were normal villi adjacent to those showing marked abnormality. The authors also suggest that the severity of the changes could not be correlated with the duration of the administration of the antibiotic.

The histological changes noted in the intestinal mucosa were qualitatively similar, although much less pronounced than those found in patients with idiopathic steatorrhea. These changes indicate that neomycin produces a definite histological alteration in the normal jejunal mucosa. It should also be noted that in the one subject who was biopsied again 18 days after cessation of the treatment the intestinal mucosa had returned to normal.

While these investigations indicate that neomycin produces a definite change in intestinal mucosa, they neither indicate the mechanism by which the alteration is produced nor prove that the malabsorption is definitely dependent upon the changes noted. The authors believe that it is highly probable that these intestinal changes, because of their similarity to those occurring in other malabsorption syndromes, are responsible for the defects in absorption, rather than the drug decreasing intestinal absorption of nutrients by merely increasing intestinal transit time.

PEMMICAN

An emergency ration of pemmican provides 1000 calories a day; adaptation to resulting starvation persists for at least a week and reduces physiological disturbances during a second starvation period.

The North American Indian prepared pemmican by drying thin slices of buffalo meat or venison in the sun. The dried meat was then pounded into small particles, mixed with melted fat, and packed in sacks of hide. Some of the early explorers used a similar preparation made of dried beef, suet, raisins, and sugar. The high caloric density and its preservative qualities are the primary reasons why pemmican has been proposed as an emergency ration for a variety of military personnel. This suggestion has elicited much discussion, but has produced relatively little experimental data on the physiological effects of eating pemmican.

The United States military forces have recently added pemmican to one of their emergency survival rations, hence, a number of reports have appeared on the reactions of military personnel to pemmican.

E. P. Torrance and R. Mason (*Am. J. Clin. Nutr.* **5**, *176 (1957)*) of the Survival Research Field Unit at Stead Air Force Base in Nevada provided each of 543 airmen with a double issue of the emergency survival ration (containing pemmican) plus two pounds of beef and a small quantity of vegetables. The food was to last for a nine-day simulated survival exercise, four days of which were spent in rest and the last five in travelling over difficult terrain.

At the end of the field test each man's reaction to the pemmican was secured by a questionnaire. The 27 per cent of the sub-

jects showing the greatest aversion to the pemmican were studied further to determine the basis for their aversion. The men who found pemmican unacceptable had been exposed to unfavorable comments about it either prior to or during the test. In a few cases the men had been too hungry or too tired when they first ate pemmican, and these conditions appeared responsible for their dislike.

A more extensive evaluation of pemmican as an emergency ration was made in Alaska by H. F. Drury, D. A. Vaughan, and J. P. Hannon (*Arctic Survival Rations, III. The Evaluation of Pemmican Under Winter Field Conditions, Arctic Aeromedical Laboratory, January (1959)*). Ten young men were restricted to a remote part of Ladd Air Force Base. They were provided with standard air force arctic clothing and double sleeping bags. During the day the men were physically active for at least six hours. They slept in unheated snow shelters.

The temperatures recorded at the camp in the morning decreased from +14°F on the first day of the test to −55° on the fifth day. From the fifth day to the ninth day the temperature gradually increased to +13°. In spite of the severity, the men did not suffer from the cold.

Each man received 1000 calories per day consisting of 5 ounces of pemmican and a number of opaque gelatin capsules. The capsules for half of the men contained pemmican; capsules for the other men contained sucrose to provide 40 g. per day. Both groups were instructed to take the capsules at regular intervals throughout the day.

Most men ate the pemmican at regular mealtimes, often with distaste, especially from the third to the fifth day. Uneaten pemmican was weighed.

Difficulty in eating pemmican was usually associated with nausea. The nausea, however, disappeared after the fifth day, then all the pemmican was eaten. The investigators suggested that the nausea was probably due to the starvation of the men as a result of the 1000 calorie allowance per day.

During the nine days of the study the men became weaker and tired easily when doing any physical activity. Resting however, produced prompt recovery, and the men could work for another short time.

The men lost an average of 10.5 pounds during the nine-day trial. There was no difference in the weight lost by the group receiving the sugar supplement and the group receiving only pemmican. The sucrose supplement increased the fasting blood sugar to only an insignificant extent (68 mg. per 100 ml. versus 64 mg.). The two groups also showed no difference in nitrogen balance as measured by nitrogen intake and urinary excretion. The sugar had no subjective response that could be detected by the observers.

The urinary excretion of acetone provided no definitive evidence for any beneficial effect of the sucrose supplement. One of the subjects receiving the sugar excreted 31 g. of acetone equivalents during the nine days, whereas the other four members of this group averaged 7.7 g. Disregarding the subject showing the highest acetone excretion, the group receiving the sugar would then show a slightly lower acetone excretion than the five men receiving nothing but pemmican (7.7 g. versus 12.4 g.).

The average daily excretion of acetone by both groups showed a peak on the third day of the study, after which the excretion values gradually returned close to normal. The nitrogen balance data also showed that the maximum loss occurred on the third or fourth day followed by a gradual return to equilibrium. There is no explanation for the apparent adaptation that occurs after the third day of starvation. Drury and co-workers observed a similar indication of an adaptive process in sedentary subjects given a 1000 calorie diet while living in a heated laboratory.

In an extension of these studies, D. A. Vaughan and co-workers (*Arctic Survival Rations, VI. The Physiological Effects of Restricted Diets During Successive Winter Field Trials, Arctic Aeromedical Laboratory,*

August (1959)) subjected 12 young men to two test periods of five days each, separated by one week, during which the men ate at home on an ad libitum basis. In both trials each man received 1000 calories per day. All subjects were given gelatin capsules as in the previous study. Four of the men had pemmican in their capsules, another four received 40 g. of sucrose per day, and the last four received 80 g. of sucrose per day.

The results of the first five-day trial were essentially the same as those noted in the previous study. The 80 g. sucrose supplement had no greater effect than the 40 g. on fasting blood sugar levels, nitrogen balance or urinary acetone excretion.

In the second trial, all subjects showed evidence of an adaptation to starvation in so far as the above parameters are concerned. All three groups showed a less precipitous drop in fasting blood sugar values during the second trial than during the first trial. Actually, the group receiving 80 g. of sucrose showed no change in blood sugar levels when compared to the pre-test control values. A similar trend appeared in the nitrogen balance values. During the first trial, the men showed a sharp increase in the nitrogen lost from the body with a peak value of 7 g. per day being reached on the second or third day. In the second trial, the nitrogen loss never exceeded 4 g. per day.

The acetone excreted in the first five-day period was inversely related to the sucrose supplement, and all values were approximately twice those observed in the second trial.

The difference in the results of the first and second trials was not attributable to either the external temperature or the physical condition of the men. The latter was evaluated by R. E. Johnson, L. Brouha, and R. C. Darling (*Rev. canad. de biol.* **1,** *491 (1942)*).

It was suggested by Vaughan and coworkers that "a metabolic adjustment or adaptation to restricted caloric intake" was developed during the first trial which persisted during the one week of rehabilitation. This concept agrees with the observation of H. L. Taylor *et al.* (*Am. J. Physiol.* **143,** *148 (1945)*) who found that men without food for a period of four days carried over an adaptation to starvation. When the men were subjected to a subsequent starvation as long as six weeks after the first starvation, they showed a reduced excretion of nitrogen and ketone bodies in the second test.

These studies suggest that the acceptance of a survival ration, which of necessity must be an unusual diet, can be enhanced by prior consumption of the ration. The work re-emphasizes the fact that metabolic and physiological adaptations to semistarvation can occur. Whether this adaptation is also associated with psychological adjustments which permit the individual to withstand the rigors of a reduced food intake is not apparent from these reports. Additional work in the latter area would certainly be desirable and might offer suggestions as to how an obese individual might best adapt himself to the rigors of a reducing diet.

TOXICITY OF VITAMIN K SUBSTITUTES IN PREMATURE INFANTS

Another article pointing out the potential hazards from the use of large doses of certain water-soluble vitamin K substitutes in young infants.

During recent years a number of authors have reported untoward effects from large doses of certain vitamin K substitutes given to prematurely born infants (a large dose is 10 mg. or more, about 1000 times that required as prophylaxis for the newborn).

These effects of hyperbilirubinemia, mild hemolytic anemia, increased frequency of Heinz-bodies in erythrocytes, and an increased incidence of kernicterus (*Nutrition Reviews* **15.** *331 (1957)*) are largely limited

to the newly born infant, especially the premature infant.

A recent study in this field by Holger Dyggve (*Acta Paediat.* **49,** *230* (*1960*)) is an extension of earlier observations (*Danish Med. Bull.* **6,** *19* (*1959*)) that prematurely born infants weighing less than 2000 g. at birth developed higher levels of serum bilirubin upon administration of menadione sodium bisulfite than those given a comparable dose (10 mg.) of vitamin K_1. No differences could be detected in those infants weighing more than 2000 g. at birth.

In the present study a larger number of subjects were available. One group of 135 prematurely born infants received 10 mg. of menadiol sodium diphosphate (Synkavit) immediately after birth. A second group of 109 babies received 10 mg. of vitamin K_1, and a third group of 73 babies were given 1 mg. of vitamin K_1. The injections were given intramuscularly; the vitamin K_1 was in aqueous suspension.

Care was taken to exclude from the study all cases of blood group sensitization and all babies whose mothers had received vitamin K preparations prior to delivery. Most babies were fed human milk; drugs known to produce hyperbilirubinemia in young babies were not given. Determinations of total serum bilirubin blood hemoglobin, and reticulocyte counts were made on alternate days during the first 12 to 13 days of life; determinations of prothrombin times were made at less frequent intervals.

Classified on the basis of birth weight, there were 180 babies who weighed between 2000 and 2500 g. The average serum bilirubin level was somewhat higher in the menadiol group than in the vitamin K_1 group throughout the 13-day period, although the differences were statistically significant only on the ninth and thirteenth days. The highest levels were recorded on the fifth day of life, and these were 14 mg. per 100 ml. for the menadiol group versus 11 mg. per 100 ml. for those receiving vitamin K_1.

The difference was more striking in those babies weighing less than 2000 g. at birth (it is regrettable that no further subdivisions of birth weight were made). Here, the average peak level for the menadiol group was 17 mg. per 100 ml. on the fourth to sixth day of life in contrast to 13 mg. per 100 ml. for the vitamin K_1 group. Similarly, the menadiol group showed higher bilirubin levels throughout the entire period of observation. The difference was statistically significant.

It is interesting that there were no differences noted between those given 1 mg. and 10 mg. of vitamin K_1. When the results were tabulated on the basis of the number of infants whose maximum level of serum bilirubin exceeded 20 mg. per 100 ml, the following was apparent: of 130 babies given 10 mg. of menadiol, 19 per cent had such a rise; the incidence was 12 per cent for the 73 babies given 1 mg. of vitamin K_1; and 8 per cent for the 106 babies given 10 mg. of vitamin K_1.

Twin infants given menadiol developed a hemolytic anemia which required blood transfusion. No cause for this was ascertained. With this possible exception, the three groups of babies were identical in all respects other than hyperbilirubinemia. No differences in blood hemoglobin concentration, reticulocyte counts, prothrombin times, or the frequency of hemorrhages during life or at post mortem examination could be detected.

Unfortunately, the author does not mention other such pertinent matters as death rate or the presence or absence of kernicterus—which frequency is definitely increased when the bilirubin level exceeds 20 mg. per 100 ml. Nor is the possibility of sex differences explored.

However, it is apparent from this study that vitamin K_1 is well tolerated by the prematurely born infant, and that the frequency of hyperbilirubinemia is increased when either menadione or menadiol is given in a dose of 10 mg. Further docu-

mentation shows that it is the very young and immature infant who is most susceptible to the toxic potentialities of these agents.

The author, in his introduction, reviews the pertinent literature on the toxicity of these compounds in animals and man and makes a plea for the use of vitamin K_1 rather than water-soluble substitutes in the prematurely born infant. Certainly the dose should be kept low if one of the latter is to be given. The recent introduction of an aqueous suspension of vitamin K_1 removes one of the objections to its intramuscular use and leads one to seriously question the need for water-soluble substitutes in this age group.

CHLORPROMAZINE TRANQUILIZATION DOES NOT INCREASE MILK PRODUCTION

Chlorpromazine fed to dairy cattle for periods up to 24 weeks, at levels several times those producing tranquilization in other species, was without significant effect on Holstein cows or on milk production.

One of the most common beliefs of dairymen is that the lactating dairy cow responds to environmental conditions by alterations in milk production. Consequently, the possibility of using a tranquilizing agent in the diet to prevent cattle from reacting unfavorably to changes in environment has been investigated by a number of dairy scientists.

There have been conflicting reports on the effects of tranquilizers on weight gain of beef calves, dairy calves, and lambs. A very interesting report was made by A. B. Hoerlein and C. L. Marsh (*J. Am. Vet. Med. Assn.* **131,** *227 (1957)*) that calves injected with chlorpromazine at the time of weaning were less disturbed and gained more weight in the week following weaning than the untreated controls. Other investigators have found little or no response to other tranquilizers used under similar circumstances. S. F. Scheidy and K. S. McNally (*Cornell Vet.* **48,** *331 (1958)*) and D. C. Jordan and G. M. Ward (*J. Dairy Sci.* **41,** *1485 (1958)*) reported that injections of a tranquilizer for several days after parturition permitted the introduction of first-calf heifers to the milking routine with a minimum of disturbance and nervous reaction.

This use of tranquilizers on cattle suggested the possibility of their use in feed, thereby reducing the nervous reaction to milking and thus increasing milk production. This led L. J. Bush, M. G. Yang, and G. V. Odell (*J. Dairy Sci.* **43,** *1118 (1960)*) to experiments in which various levels of the tranquilizer were included in the diet of lactating Holstein cows.

Chlorpromazine hydrochloride was included in the grain ration, and the daily allowance (ranging from 0.05 to 5.0 g.) was divided into two equal portions and given at the morning and evening feedings. Bush, Yang, and Odell were careful to check the possible transfer of the tranquilizer to milk, but they were unable to detect the presence of the chlorpromazine or its principal metabolite, chlorpromazine sulfoxide, either in the milk or in the blood of the cows receiving the highest levels of chlorpromazine in the diet.

They were not able to detect the presence of the drug even when much higher levels (up to 9.0 g.) of chlorpromazine were administered as a single dose. Interestingly enough, Bush, Yang, and Odell were unable to detect any effect upon milk production over a 24-week period. They concluded, therefore, that no alteration in milk production developed and that no tranquilization was apparent after daily administering chlorpromazine at levels up to 5 g.

Thinking that adverse environmental

conditions might accentuate the effect of the tranquilizer, Bush, Yang, and Odell placed cattle on three different levels, 0, 250, 500 mg. per day, exposing half of them to the environmental conditions existing in Oklahoma between November 1 and April 17, without any protection, and allowing the other half the protection of an open-end shed. They could detect no statistical differences in the milk production of these cattle.

It was apparent from these results that the use of chlorpromazine would not result in significantly increased milk production in lactating Holsteins. Tranquilization, however, was evidenced after doses of 4.5 and 9.0 g. of chlorpromazine at a single feed. Significantly, with this high level of chlorpromazine, they were unable to detect the drug in the cows' milk or blood.

This interesting report of the effect of a tranquilizer drug shows that long-time feeding of the drug has little prospect of increasing milk production and that levels much higher than those ordinarily used in other species exert no tranquilizing effect. Even more significant, when the drug is used in veterinary practice, is that tranquilizing levels of the drug are not secreted in the milk.

LYSINE AND THE ABSORPTION OF CALCIUM

Lysine markedly increased the absorption of calcium from the ileum of fasted rats, but had no effect when added as a supplement to a normal diet.

A number of years ago it was observed that alpha-amino acids increase the solubility of calcium (H. Lehmann and L. Pollak, *J. Physiol.* **100,** *17 (1941)*). This fact, plus the fact that poor absorption of calcium occurs in diets deficient in protein (R. A. McCance, E. M. Widdowson and Lehmann, *Biochem. J.* **36,** *686 (1942)*), suggested that the amino acids might have a direct effect on the absorption of calcium.

To test this possibility, R. H. Wasserman, C. L. Comar and M. M. Nold fed male rats (90 to 130 g. body weight) radioactive calcium and one of the essential amino acids. When the rats that had been fasted for 22 hours were given the preceding substances by stomach tube, lysine and arginine increased the absorption 1.7 to 1.8 times that of the control (no amino acid). Lysine was 80 per cent as effective as lactose in increasing the absorption of calcium. The absorption was determined by measuring the radioactivity of the femurs removed from the animals 24 hours after the test dose was administered.

In the above work, the amino acids, if given parenterally, had no effect on the absorption of calcium. From this, the investigators concluded that the lysine influenced the absorption of calcium from the intestinal tract and not through any action which might be associated with its deposition in bone.

As an extension of this work, A. M. Raven, F. W. Lengemann, and Wasserman (*J. Nutrition* **72,** *29 (1960)*) attempted to determine the segment of the intestinal tract in which lysine exerted its effect. To do this, they injected 5 μc of calcium^{45} and 0.84 millimoles of lysine in 1 ml. of solution directly into the lumen of ligated sections of the intestinal tract. The effect of the lysine was studied by measuring the radioactivity in the femur five hours after the material was injected. Lysine increased the absorption of calcium only in the ileum. It had no effect on the absorption from the stomach, duodenum, jejunum or colon.

The lysine was active in enhancing the absorption of the mineral only when the two substances were present in the same segment simultaneously. In most of these studies strontium^{85} was present with the calcium in the test solutions. The absorption of both

minerals was influenced to the same extent by the substances under test. Strontium was used to determine how quickly the effect of the amino acid on the absorption of the mineral could be detected. Lysine had a detectable effect as early as 30 minutes after the start of the experiment and was very pronounced after one hour.

The addition of lysine as a supplement to a diet had no effect on the absorption of calcium or strontium, as measured by whole body retention. In these studies, lysine at levels of 1, 2 and 4 per cent was added to a basal diet of purified ingredients. Rats weighing 80 g. were maintained on the diets for 14 days. The isotopic calcium and strontium were provided in the drinking water.

Since lysine increased the absorption of a single dose of isotopic calcium and strontium when fasted rats were used and since it had no effect when tested in a complete ration, efforts were made to determine which ingredients inhibited its action. When fasted rats were given a single dose of isotopic calcium and strontium supplemented with lysine and either histidine or glutamic acid, the enhancing effect of lysine was largely overcome. Glutamic acid and arginine had no effect on the action of the lysine. In the preceding studies the lysine and either of the other amino acids were present in equal molar concentrations. Similar experiments carried out with lysine and casein showed that the protein depressed the action of the lysine.

Other studies indicated that the effect of lysine in enhancing calcium absorption was most marked when the level of calcium in the test dose was relatively low (3.6 mg. of calcium) and the amount of lysine on a molar basis was 13.9 times that of the calcium. As the amount of calcium was increased, the effect of the lysine decreased to zero with 36 mg. of calcium in the test solution.

These studies show that although lysine is effective in increasing the absorption of both calcium and strontium from the intestinal tract of the rat, it does so only when the amino acids and minerals are fed to fasted rats. The presence of other dietary ingredients markedly decreases the lysine effect. Under these circumstances, Raven *et al.* suggest that lysine enhances the absorption of calcium and strontium in the single-dose studies by a direct action on the gut wall.

These studies emphasize the fact that some substances which markedly influence the absorption of other nutrients under specialized and restricted experimental conditions may have no effect under normal dietary practices. For this reason, it is especially important to study the effect of the suggested enhancing substances under normal feeding practices.

RELATION OF PANTOTHENIC ACID TO ADRENAL CORTICAL FUNCTION

The adrenal cortex of healthy rats contains free pantothenic acid in a rather uniform distribution. After stimulation with ACTH, the concentration of coenzyme A increases selectively in the zone fasciculata at the expense of free pantothenic acid.

Recently, the relationship between pantothenic acid and adrenal cortical function has been reviewed (*Nutrition Reviews* **18,** *273 (1960)*). Evidence has been presented to indicate that animals deprived of pantothenic acid or given a large amount of the antagonist omega methyl pantothenic acid become incapable of forming normal quantities of adrenal cortical steroids. Pantothenic acid deficiency in rats leads to depletion of adrenal cholesterol (R. W. Winters, R. B. Schultz, and W. A. Krehl, *Proc. Soc. Exp. Biol. Med.* **79,** *695 (1952)*). Administration of adreno-corticotrophic hormone (ACTH)

to such animals hastens the depletion of cholesterol, whereas cortisone restores it to normal (Schultz, Winters, and Krehl, *Endocrinology* **51,** *336 (1952)*).

It has been suggested that the adrenal hemorrhagic necrosis associated with this deficiency might be due to disruption of the balance between ACTH and cortisol resulting in an excessive stimulation of defective adrenal glands. However, L. C. Butler and A. F. Morgan (*Endocrinology* **56,** *322 (1955)*) found that the quantities of ACTH in the pituitaries and in the venous blood of pantothenic acid-deficient female rats were similar to those of control animals. Obviously the complete answer is not yet available.

In an attempt to study this problem further, R. M. Twedt and D. Glick (*Endocrinology* **67,** *547 (1960)*) employed a quantitative histochemical technique to measure the distribution of free and bound pantothenate in various zones of rat adrenals under normal conditions and following stimulation with ACTH. They employed white male rats weighing 350–450 g. ACTH was given to some of the animals in a dose of 25 mg./kg. three hours before sacrifice. The animals were sacrificed quickly and the left adrenal gland was removed and frozen immediately with carbon dioxide snow. These frozen glands were sectioned with a microtome to allow estimates of the concentration of pantothenic acid in each zone of the cortex. Additional sections were taken for histological examination and for protein nitrogen determination. Measurements were made by means of biological assay systems of both the free and bound pantothenic acid in the different zones of the adrenal cortex.

It had been found, by chemical means, that in the normal animals there was more coenzyme A in the outer portion of the zona fasciculata and in the border between the fasciculata and reticularis. In the present study the authors assumed that bound pantothenic acid was similar to coenzyme A and provided a rough estimation of this substance. They found that throughout the adrenal cortex less than 20 per cent of the total pantothenic acid was present in the free form and that free pantothenic acid was uniformly distributed throughout. However, following treatment with ACTH there was a reduction of free pantothenic acid in all zones and a rather marked increase of bound pantothenic acid in the zona fasciculata. This apparently was due to a conversion of the free pantothenic acid to the bound form of the vitamin, presumably coenzyme A.

The authors concluded that ACTH increased the concentration of coenzyme A selectively in the zona fasciculata. No doubt it is more than coincidence that Schultz *et al.* found this to be precisely the region of the adrenal cortex from which cholesterol was removed most rapidly in pantothenic acid-deficient animals, and it also was the portion of the gland most susceptible to hemorrhagic adrenal necrosis.

While this report does not answer the problem of the interrelationships between coenzyme A, ACTH, and adrenal cortical function, it does provide useful information to suggest that the zona fasciculata is the most active functional portion of the gland when stimulated by ACTH, and it further suggests that pantothenic acid, or its derivative coenzyme A, also has its greatest metabolic activity in this zone.

COPPER IN CYTOCHROME C OXIDASE

New evidence suggests that in addition to containing heme, cytochrome c oxidase also contains cuprous ions bound to protein.

The controversy as to whether cytochrome *c* oxidase is a copper-containing enzyme or an iron-containing enzyme has existed for more than 20 years. Nutritional studies dating from the early 1930's to recent years have repeatedly shown marked re-

ductions in the cytochrome oxidase content of tissues of copper-deficient animals (*Nutrition Reviews* **14,** *281 (1956)*). This, however, is only circumstantial evidence that the enzyme contains copper, and the decrease has many times been explained by an effect of copper upon the incorporation of iron into the enzyme, specifically the heme moiety of the enzyme. A number of studies on cytochrome oxidase preparations have indicated that cytochrome oxidase must be an iron-porphyrin-protein complex (D. Keilin and E. F. Hartree, *Proc. Roy. Soc. London B* **127,** *167 (1939)*; P. Person, W. W. Wainio, and B. Eichel, *J. Biol. Chem.* **202,** *369 (1953)*), while other studies have suggested that the oxidase is a copper-containing enzyme (Eichel, Wainio, Person, and S. J. Cooperstein, *J. Biol. Chem.* **183,** *89 (1950)*). The point not generally realized in the early work was that it might contain both iron and copper and that the copper need not necessarily be bound to the porphyrin moiety.

Recent investigations have indicated that sub-mitochondrial preparations high in cytochrome *c* oxidase activity invariably have a high copper content (D. E. Green, R. E. Basford, and B. Mackler, *A Symposium on Inorganic Nitrogen Metabolism, p. 125*, W. D. McElroy and H. B. Glass (*ed.*), *Johns Hopkins Press, Baltimore (1956)*; K. Okunuki *et al., Proc. Int. Symposium on Enzyme Chemistry, 1957, p. 264, Tokyo-Kyoto, Maruzen Co. Ltd., Tokyo (1958)*). Wainio, C. Vander Wende, and N. F. Shimp (*J. Biol. Chem.* **234,** *2433 (1959)*) now claim that cytochrome *c* oxidase is in fact a copper-containing enzyme which also contains heme and that the copper exists in the cuprous state probably bound to protein (Vander Wende and Wainio, *J. Biol. Chem.* **235,** *PC11 (1960)*).

The major difficulty in ascertaining the constituents of cytochrome oxidase has been the insoluble nature of the enzyme, thereby greatly hindering its purification. Wainio *et al.* (*J. Biol. Chem.* **234,** *2433 (1959)*) fractionated the "insoluble heart muscle preparation" containing cytochrome *c* oxidase by adding 1 per cent (or sometimes 0.5 per cent) of sodium desoxycholate, which tends to "solubilize" the particulate fraction. By centrifuging at first for one hour at 144,000 $\times$ *g* and subsequently re-extracting with desoxycholate and centrifuging for half-hour intervals, they obtained a series of "solubilized" fractions and a residue. These fractions (three to seven in various experiments) were analyzed for protein, copper, cytochrome oxidase-heme (calculated from 605 mμ absorption) and in one of the reported experiments for cytochrome *c* oxidase activity. About 55–70 per cent of the protein of the particulate material could be solubilized before the heme (605 mμ. absorption) became soluble. The copper, as measured by copper protein ratio, was concentrated in the fractions possessing the greatest amount of heme per unit of protein. The copper-heme ratios in these fractions, however, varied from 0.8 to 3.2. It was clear that the copper and cytochrome oxidase-heme tended to be solubilized together; however, in view of the variable copper-heme ratios, some of the copper or the heme as measured were probably attributable to substances other than cytochrome *c* oxidase.

In one experiment six soluble fractions and the residue were prepared and cytochrome *c* oxidase activity was measured in addition to the protein, copper, and cytochrome oxidase-heme content. The authors found, in agreement with previous work, that a "lipid soluble substance" which occurred in the fraction most easily solubilized by desoxycholate was necessary to "activate" the other fractions. This "activation" was much greater for some fractions than for others. Although the cytochrome *c* oxidase activities did not correlate well with the copper or heme contents of the fractions when the "lipid factor" was omitted, there was a relationship between copper, heme, and cytochrome *c* oxidase activity when the "activator" was added. The correlation between copper, heme, and enzyme activity, although impressive, was not perfect as indicated by sizeable variations in cytochrome

oxidase activity-heme and cytochrome oxidase activity-copper ratios. Again, this may indicate the occurrence of heme and/or copper in constituents other than cytochrome oxidase.

In the further studies of Vander Wende and Wainio (*J. Biol. Chem.* **235,** *PC11 1960*)) copper was removed from solubilized cytochrome *c* oxidase preparations by dialysis against various solutions containing copper complexing agents. The complexing agents were subsequently removed by further dialysis against water. A clear cut parallel between the percentage of copper retained by the preparation and the cytochrome oxidase activity resulted. When all of the copper was removed (dialysis against 0.1 molar sodium diethyldithiocarbamate), no oxidase activity was detectable. For the cytochrome oxidase preparations which were dialyzed against three different concentrations of sodium cyanide, the iron content was shown not to decrease with the copper and the cytochrome oxidase activity.

Attempts to regenerate the cytochrome oxidase activity by adding copper ions resulted in variable success. Sources of cupric ions were ineffective, although the preparations did bind copper. Inclusion of ascorbic acid during regeneration to reduce the copper ions to the cuprous form resulted in nearly complete restoration of activity when copper was originally removed by diethyldithiocarbamate, but usually only slight reactivation when the copper was removed with cyanide. Possibly residual cyanide acted as an inhibitor. The amount of copper bound by the enzyme preparations during regeneration was highly variable. Using chelating agents specific respectively for Cu^{+} and Cu^{++}, it was demonstrated that the copper in cytochrome *c* oxidase preparations was almost entirely in the reduced state even in air-oxidized preparations, which suggests that alternate oxidation and reduction of copper may not occur during electron transport. While the site at which copper is bound is not known, Vander Wende and Wainio suggested that, since the pyridine-extractable hemochromogen prepared from cytochrome oxidase preparations contains no copper and chymotrypsin and trypsin liberate about 40 per cent of the copper from the enzyme preparations, the copper is probably bound to protein.

The authors have concluded that cytochrome *c* oxidase is a copper-containing enzyme which also contains heme and that the copper is primarily in the cuprous state bound to protein. Probably their best evidence is the loss of enzyme activity upon removing the copper and its subsequent regeneration with cuprous ions. However, until cytochrome oxidase is isolated and studied in pure form and the stoichiometric amounts of copper are determined, the status of copper in cytochrome *c* oxidase preparations will remain open to question. The present interpretations, however, most directly explain the marked effect of dietary copper on the cytochrome oxidase content of animal tissues.

BODY FAT AND LACTOSE FEEDING

Rats fed a diet containing lactose as the carbohydrate source have a decreased percentage of carcass fat and an increased weight of the cecum.

Various investigators have noted that both rats and pigs maintained on diets containing lactose as the sole carbohydrate have carcasses with a lower fat content than animals fed diets containing glucose. The reason for this difference is not known though it is well established that excessive amounts of lactose in the diet of rats lead to toxicity. The symptoms of this disorder are diarrhea, enlargement of the cecum, unthrifty appearance, alopecia, and growth retardation.

Recently, R. M. Tomarelli, R. Hartz, and F. W. Bernhart (*J. Nutrition* **71,** *221 (1960)*) have re-investigated this problem using rats

as experimental subjects. Two groups of seven animals each were maintained for six weeks on diets containing 24 per cent casein, 52 per cent carbohydrate with the usual amounts of minerals, fats, and vitamins. The carbohydrate in the diet of one group was glucose and in the other group was lactose. The third group received glucose as the carbohydrate, but the food intake was limited to that ingested by the lactose-fed group (paired feeding).

The animals which ate the lactose diet grew at a rate of about 70 per cent of those fed glucose, with the average weight for the two groups being 238 and 309 g., respectively. The bodies of the animals fed the glucose-containing diet contained 14.3 ± 0.14 per cent fat, while those maintained on the lactose diet had only 8.3 ± 0.2 per cent. Since the animals on the lactose-containing diet grew slower than the controls fed glucose, a second group of animals receiving glucose was pair fed with the lactose group. These latter glucose animals weighed on the average of 256 g., which was greater than the weight of those getting lactose, but less than those receiving glucose ad libitum. However, the fat content of the carcasses of the glucose-fed animals was not different from the controls (14.4 ± 0.9 per cent fat).

The protein content of the carcass of the three groups of animals was not different, though a higher ash content was found in the carcass of those getting lactose than in the animals fed glucose (3.8 ± 0.1 as compared to 3.3 ± 0.1 per cent). Whether this was due to an increased calcium content was not reported.

To determine whether these changes were due to the intact lactose or possibly due to the components of lactose (glucose and galactose), a study was carried out in which groups of animals received as their carbohydrate either 52 per cent lactose, 26 per cent glucose plus 26 per cent galactose, or 52 per cent glucose. The animals getting the galactose in their diet grew slower than the other two groups and had a significant decrease in the carcass fat. However, there was no difference in carcass fat between the animals receiving the glucose plus galactose or the glucose alone. The animals which ate the glucose-containing diet grew some better than those receiving the glucose plus galactose. The weight of the cecum of the animals receiving lactose was about three times that of those receiving the other two sugars.

Thus, it appears that the low carcass fat is related to events occurring in the gastrointestinal tract. It was found that the pH of the cecum in the animals fed lactose was 6.52; when the animals were getting galactose plus glucose or glucose alone, the pH of the cecum was about 7.9. Feeding sorbitol, cellobiose or raw potato starch resulted in a decrease in the pH of the cecal contents, an increase in the weight of the cecum, and a decrease in the carcass fat.

While the mechanism by which lactose feeding influences the body fat composition is not known, there appears to be a relationship between the intestinal flora and the change in fat composition of the body. These investigators suggest that the lactose feeding, in addition to producing an enlarged cecum, caused a conversion of the microflora in the cecum to one which is predominantly gram positive. The effect of changing microflora on the growth characteristics of animals has been reviewed (*Nutrition Reviews* **19,** *12 (1961)*) and indicates the actual cause of the body changes.

EXPERIMENTAL HEMOCHROMATOSIS

The experimental counterpart to human pigment cirrhosis has been produced in rats by feeding a choline-deficient diet supplemented with extra iron.

Several techniques have been used in attempts to produce hemochromatosis in experimental animals. Diets have been given which promote iron absorption, and toxic

substances have been injected or various forms of iron have been given to increase iron deposition in many tissues of the body. Pigment cirrhosis is characteristic of the human disease, but it occurs rarely, if at all, in these experimental preparations. In the present experiment, R. A. MacDonald (*Am. J. Path.* **36,** *499 (1960)*) used a choline-deficient diet with added iron followed by correction of the deficiency. Apparently, under these conditions he was able to produce hepatic cirrhosis analogous to the human pigment cirrhosis.

In a study of human subjects MacDonald and G. K. Mallory (*Arch. Int. Med.* **105,** *686 (1960)*) enumerated several findings indicating that nutritional deficiency is involved in the pathogenesis of pigment cirrhosis: (a) hemochromatosis occurred with unusual frequency in a population which also had many cases of cirrhosis related to malnutrition and alcoholism; (b) the livers of patients with hemochromatosis also had residual fat and "alcoholic hyalin" suggesting an early alcoholic or nutritional cirrhosis; (c) patients with alcoholism were frequently found to have hemosiderosis; (d) lack of well-defined pathological or clinical differences in patients with hemosiderosis, advanced hemosiderosis, and hemochromatosis suggested that they were all gradations of a single disease.

Two experiments were performed in the present investigation. Experiment I was designed to determine whether a diet which produced cirrhosis and fatty liver would also increase iron absorption and whether removal of the deficient diet would produce an analogue of human pigment cirrhosis. Tritiated thymidine autoradiography was used to follow hepatic cell regeneration based on the extent of utilization of the thymidine in deoxyribonucleic acid synthesis. Experiment II attempted to learn whether increased iron absorption continued after removal of the choline-deficient diet.

In Experiment I, male rats, 82 days old and weighing between 200 and 300 g. were divided by littermates into two groups of eight rats each. One group was fed a choline-deficient diet containing 3 per cent ferric ammonium citrate. The second group was fed a stock diet with 3 per cent added iron. After 21 days the animals were etherized and their livers perfused, formalin fixed, part stained by ammonium sulfide or potassium ferrocyanide and part used for chemical determination of iron. In the second part of this experiment, four to seven rats were fed stock diet with and without added iron, choline-deficient diet with and without added iron, and choline-deficient diet with and without iron, but supplemented with choline. Some rats fed the choline-deficient diet with added iron for 69 and 97 days were given a stock diet for the duration of the experiment. Tritiated thymidine was injected intraperitoneally four hours prior to sacrifice. Tissues were fixed and stained using a number of special techniques.

In Experiment II, eight rats were fed a choline-deficient diet and eight control rats were fed the stock diet for 85 days followed by a 21-day feeding of stock diet to correct the previous deficiency. At this time a 0.2 g. biopsy of liver was taken for iron determination. Iron supplemented stock diet was fed all rats for 40 additional days after which the animals were sacrificed and livers stained and analyzed for iron.

The concentration of iron in the livers of animals on the choline-deficient diet in Experiment I was approximately doubled. Since the average weight of liver in the choline-deficient group was also doubled, the total amount of iron was much greater than in the control group. Feeding the choline-deficient diet alone with no added iron produced fatty liver and cirrhosis but no hemochromatosis.

Hemosiderin was found in the liver and other organs of the rats eating the choline-deficient diet with added iron. In the liver the pigment was seen mostly in the hepatic cells, but iron was also found in macrophages, fibrous tissue, endothelium, connective tissue, and in cells in all parts of the liver lobule.

Heart, pancreas, and adrenal interstitial tissue showed deposits of iron as well as stomach interstitial and parenchymal cells and proximal tubular epithelium of the kidney. Other areas showing iron deposition included interstitial tissues of mucosa, submucosa and subserosal connective tissue of the gastrointestinal tract. There were small amounts of iron deposition in alveolar cells of the lungs, large amounts about blood vessels and in interstitial cells of the testes, and large amounts in abdominal lymph nodes and spleen.

Restoration to a normal diet after eating the choline-deficient diet plus 3 per cent iron resulted in one of two patterns: in animals which had developed cirrhosis, the lesion, after fat had been mobilized from the liver, was one of portal cirrhosis with heavy deposits of hemosiderin; animals that had eaten the experimental diet poorly did not get cirrhosis. When the normal diet was substituted, fat was removed from the liver but essentially no fibrosis was present. Nevertheless, iron was deposited in the liver and other organs as before.

Animals that received the choline-deficient diet with added choline and iron still deposited iron, but the amount appeared to be less. Rats eating the stock diet with 3 per cent iron for 69 days had small deposits in hepatic and Kupfer cells and other areas, but larger deposits in lymph nodes and in the pulp of the spleen. Heavier deposits were found after 156 days. Stock diet fed without added iron resulted in hemosiderin deposits only in the pulp of the spleen. Study of thymidine utilization and hepatic cell mitotic activity did not reveal any increase in regeneration resulting from the increased iron. Feeding the choline-deficient diet either with or without added iron resulted in fatty liver and a marked increase in regenerative activity. Restoration of activity to normal occurred when rats fed choline-deficient diets were shifted to the stock ration.

In Experiment II, rats fed a choline-deficient diet for 85 days, then given a stock diet for 21 days, did not tend to absorb and deposit more dietary iron when fed an iron-containing diet than did animals previously on a normal ration. This indicated the lack of an irreversible change in the liver or gastrointestinal tract which would lead to continuation of iron absorption and deposition.

The rats described in this investigation showed several differences from the human lesions of pigment cirrhosis. Bile duct epithelium did not show granules of hemosiderin nor did iron appear in the pancreatic acinar cells before large quantities had accumulated in the adjacent interstitial tissues. Pancreatic fibrosis was not observed. The cytoplasm of the myocardium was also free of iron. The author attributed these differences to the short duration of the experiments. He further proposed that study of changes in serum iron content and binding capacity under these experimental conditions might furnish information relevant to the better understanding of the human disease.

COBAMIDE COENZYMES

Coenzymes of the vitamin B_{12}-group of substances containing an adenine-sugar moiety in place of cyanide have been isolated and their significance in microorganisms and animals demonstrated.

Identification of coenzyme forms of the various B-vitamins and their role in enzymatic reactions has been one of the outstanding contributions of modern biochemical research. Such knowledge is quite extensive for most of the B-vitamins, however, it is largely through the recent work of H. A. Barker, H. Weissbach, and associates that knowledge of coenzyme forms of B_{12} has been increased.

Barker *et al.* have expanded their earlier brief reports (*Proc. Nat. Acad. Sci.* **44,** *1093 (1958)*; **45,** *521 (1959)*). Most of their work has been done with microorganisms, but their discoveries in animal metabolism are also significant. Barker *et al.* (*J. Biol. Chem.*. **235,** *181 (1960)*) first isolated from the microorganism, *Clostridium tetanomorphum*, a coenzyme which they called adenylcobamide coenzyme. They found that the major route of metabolism of glutamate by this organism was via beta-methylaspartate to mesaconate, a route distinct from the known tricarboxylic acid cycle mechanism.

These reactions involve a rearrangement of the carbon atoms and a loss of ammonia. The first step (rearrangement from glutamate to beta-methylaspartate) was shown to require a coenzyme adsorbed by charcoal. Barker and co-workers developed assay systems to follow purification of this factor. The most useful one, especially during later stages of purification, was based on the absorption of light by mesaconate at 240 mμ. Charcoal treated preparations from *C. tetanomorphum* were used in the assays as the source of apoenzyme. By a series of steps using warm ethanol extraction, ion exchange chromatography, and partition between phenol and water and between phenol and ether or acetone, they were successful in preparing from *C. tetanomorphum* highly purified, but not crystalline, coenzyme.

Spectral studies, especially of the cyanide treated coenzyme, demonstrated marked similarities with the vitamin B_{12} group of substances. The coenzyme had a biological activity for *Escherichia coli* mutant 113-3 approximately equal to that of cyanocobalamin (vitamin B_{12}) and three times as great as that of pseudovitamin B_{12} (contains adenine in place of dimethylbenzimidazole). However, the coenzyme was completely inactive for *Ochromonas malhamensis*, which responds to vitamin B_{12} but not to pseudovitamin B_{12}. The coenzyme contained unimolar quantities of cobalt, phosphate, and ribose, and bimolar quantities of adenine.

Contrary to the first published report, cyanide could not be detected in the coenzyme when a more specific analytical procedure was used. Thus, the coenzyme was similar to pseudovitamin B_{12} as it contained adenine, but different in that cyanide was absent and an "extra" mole of adenine was present. They postulated that the second adenine moiety was probably bound to the cobalt atom by the coordination site often occupied by cyanide and suggested the name adenylcobamide (AC) coenzyme.

Barker *et al.* (*J. Biol. Chem.* **235,** *480 (1960)*) by similar procedures isolated cobamide coenzymes containing benzimidazole and 5,6-dimethylbenzimidazole in place of the one, ribose-bound adenine moiety (benzimidazolylcobamide (BC) coenzyme and dimethylbenzimidazolylcobamide (DBC) coenzyme, respectively). These coenzymes could be isolated in crystalline form from *C. tetanomorphum* grown in a medium containing the appropriate base, or the DBC coenzyme could be isolated from cultures of *Propionibacterium shermanii* grown in ordinary medium.

The DBC coenzyme differed from vitamin B_{12} by a lack of cyanide and by the presence of adenine. By exposure to light hydroxocobalamin was formed from the DBC coenzyme with release of an adenine-containing component. A molecular weight of 1490 for the DBC coenzyme, calculated from hydroxocobalamin (1355) plus adenine (135), differed from the molecular weight estimated experimentally (about 1660) by approximately 170. This was explained by an unknown sugar moiety of 5–8 carbon atoms attached to the "extra" adenine moiety.

Kinetic studies of the glutamate to beta-methylaspartate reaction indicated that the substrate affinity was greatest for BC coenzyme, intermediate for AC coenzyme, and least for DBC coenzyme. However, maximal activity was similar for all three coenzymes, indicating that the ribose-bound, six-

membered rings (adenine, benzimidazole, and dimethylbenzimidazole) or their substituents are not directly involved in the catalytic activity. On a molar basis biological activities of the BC and DBC coenzymes for *E. coli* and for *O. malhamensis* were similar to those of the corresponding B_{12}-vitamins.

By studying the degradation products of adenylcobamide coenzyme exposed to cyanide, acid, and light, Weissbach *et al.* (*J. Biol. Chem.* **235,** *1462 (1960)*) gained insight into the structure of the cobamide coenzymes. The authors postulated a structure for AC coenzyme which differs from that of vitamin B_{12} (*Nutrition Reviews* **14,** *64 (1956)*) by having an adenine moiety in place of dimethylbenzimidazole (as does pseudovitamin B_{12}) and by having an adenine-X moiety attached to cobalt in place of cyanide. Thus, the adenine-X moiety in place of cyanide is characteristic of the group of cobamide coenzymes. The correctness of this type of structure is supported by the preliminary X-ray diffraction studies of DBC coenzyme by C. F. T. Mattern (*J. Biol. Chem.* **235,** *489 (1960)*) and J. N. Ladd, H. P. C. Hogenkamp, and Barker (*Biochem. Biophys. Res. Comm.* **2,** *143 (1960)*). The sugar moiety of adenine-X has not been identified, but the evidence indicates that it is neither ribose nor a 2-deoxy-sugar, that it contains three vicinal hydroxyl groups, and that it probably contains at least two polar groups (*loc. cit.*).

The significance of beta-methylaspartic acid in animal metabolism has not been elucidated, but a recent report indicates that this compound, administered to pernicious anemia patients (1 g./day), caused reticulocytosis, and increased appetite and a feeling of well-being, with no increase in hemoglobin formation (E. Seifter, H. Isenberg, A. Sawitsky, and J. I. Berkman, *Abst. 138th meeting of A. C. S., p. 82C (1960)*). It had no effect on the growth of *L. leichmanii* grown on a vitamin B_{12}-deficient medium nor on growth of vitamin B_{12}-deficient rats.

More direct evidence of a role of cobamide coenzymes in animal metabolism has been obtained. The DBC coenzyme has been prepared from rabbit liver, but this was not used as a preferred source in the early work because of pronounced losses during extraction and isolation (Weissbach, J. Toohey, and Barker, *Proc. Nat. Acad. Sci.* **45,** *521 (1959)*). The interconversion of succinyl-CoA and methylmalonyl-CoA, a reaction important in the propionate to succinate interconversion and similar to the glutamate-beta-methylaspartate reaction studied by Barker *et al.*, has been shown to be vitamin B_{12}, dependent in animals (R. M. Smith and K. J. Monty, *Biochem. Biophys. Res. Comm.* **1,** *105 (1959)*) as well as in microorganisms. Furthermore, the DBC coenzyme has been shown to be active in this reaction in preparations from *Propionibacteria* (E. R. Stadtman, P. Overath, H. Eggerer, and F. Lynen, *Ibid.* **2,** *1 (1960)*) as well as from beef liver, in which case vitamin B_{12} was actually inhibitory probably due to competition with DBC coenzyme (J. R. Stern and D. L. Friedman, *Ibid.* **2,** *82 (1960)*).

These two reactions catalyzed by cobamide coenzymes can be considered as propionate transfer reactions in which a carbon-carbon bond of the beta-carbon of the propionate moiety of a straight chain structure is broken and the propionate moiety then transferred to another acceptor so that the alpha-carbon forms the new carbon-carbon bond of the branched chain product. The importance of such reactions, especially in ruminant animals which metabolize large amounts of propionate, is apparent.

The relationships of the cobamide coenzymes to the other known functions of vitamin B_{12} are less clearly indicated. J. R. Guest, C. W. Helleiner, M. J. Cross, and D. D. Woods (*Biochem. J.* **76,** *396 (1960)*) have shown with sonic extracts of *E. coli* that under the conditions in which vitamin B_{12} promotes synthesis of methionine from homocystine and serine, the DBC coenzyme

is two to three times as active as vitamin B_{12}; however, other reports show that the coenzyme is inactive in methionine synthesis (R. L. Kisliuk, *Fed. Proc.* **19,** *416 (1960)*; S. Takeyama and J. M. Buchanan, *Ibid.* **19,** *417 (1960)*). Although a specific cobamide coenzyme function has not been demonstrated in the conversion of ribosyl units to deoxyribosyl units (a reaction involving vitamin B_{12}), R. H. Abeles, H. A. Lee, Jr. and A. M. Brownstein (*Abst. 138th Meeting of A.C.S., p. 22C (1960)*) have evidence that a vitamin B_{12}-coenzyme (similar in properties to Barker's coenzymes though the structure was not described) is involved in the conversion of 1,2 propanediol to propionaldehyde by certain microorganisms, a reaction which may resemble the ribosyl-deoxyribosyl conversion. On the other hand, J. S. Dinning (*Proc. Soc. Exp. Biol. Med.* **104,** *431 (1960)*) presented evidence that vitamin B_{12} was much more effective than DBC coenzyme in stimulating *in vitro* the conversion of labeled formate to the 5-methyl group of thymine by bone marrow cells from vitamin B_{12}-deficient chicks. According to Dinning, "It appears that vitamin B_{12} has at least two distinct metabolic roles: one in reduction of one carbon compounds, and the other in biochemical rearrangements catalyzed by coenzymes first described by Barker *et al.*"

We can expect further clarification of the role of the vitamin B_{12}-coenzymes and their mechanism of action.

VITAMIN B_6 DEFICIENCY AND URINARY OXALATE

The increased urinary excretion of oxalate with consequent urolithiasis in vitamin B_6-deficient rats is accentuated by dietary glycine and by pyridoxine antagonists; it is apparently alleviated by high dietary magnesium.

The problem of renal and urinary concretions in man and animals is of complex and diversified etiology, and the basic cause of calculus formations usually is poorly understood. A predominant form of concretion in man is calcium oxalate. In the past few years primary hyperoxaluria has been recognized in man and is generally considered to be a metabolic disease. The biochemical site of the "inborn error of metabolism" has not yet been elucidated (*Nutrition Reviews* **16,** *75 (1958)*; **17,** *104 (1959)*). Whereas previous concepts associated urinary oxalates with exogenous oxalate consumed in the food, it is well recognized that in primary hyperoxaluria the source of oxalate is endogenous. The condition of primary hyperoxaluria, however, is believed to be rare and the majority of cases of oxalate stones are largely unexplained.

Important contributions to our knowledge of nutritional factors which affect urinary oxalate have recently been made independently by two research groups. S. N. Gershoff, F. F. Faragalla, D. A. Nelson, and S. B. Andrus (*Am. J. Med.* **27,** *72 (1959)*), while studying the effects of vitamin B_6 deficiency in cats, demonstrated severe oxalate nephrocalcinosis associated with increased urinary oxalate. This paper has recently been reviewed (*Nutrition Reviews* **18,** *284 (1960)*). Subsequently, this group showed in rats a marked effect of vitamin B_6 deficiency on oxalate excretion and urolithiasis (Andrus, Gershoff, and Faragalla, *Am. J. Path.* **35,** *671 (1959)*; Andrus, Gershoff, Faragalla, and E. L. Prien, *Lab. Invest.* **9,** *7 (1960)*; Gershoff and Faragalla, *J. Biol. Chem.* **234,** *2391 (1959)*).

While conducting biological assays for vitamin B_6 in food products, W. K. Calhoun, R. B. Jennings, and W. B. Bradley (*J. Nutrition* **67,** *237 (1959)*) independently ob-

served excess urinary calcium oxalate excretion with associated calculus formation in rats deficient in vitamin B_6.

Although a diffuse form of oxalate nephrocalcinosis was observed in vitamin B_6-deficient cats (*Nutrition Reviews* **18,** *284 (1960)*), when vitamin B_6-deficient rats were studied, gross calcium oxalate concretions were evident instead at the tips of the renal papillae (Andrus, Gershoff, and Faragalla, *Am. J. Path.* **35,** *671 (1959)*; Andrus, Gershoff, Faragalla, and Prien, *Lab. Invest.* **9,** *7 (1960)*). These calculi of calcium oxalate monohydrate in rats closely resembled oxalate calculi seen in man and produced all of the obstructive sequelae upon dislodgment.

The authors characterized in detail the calculi and their effects. The incidence of calculi in vitamin B_6-deficient rats fed a 15 per cent casein diet was much less (one of eight at seven weeks) than in rats fed this diet containing 3 per cent of supplemental glycine (eight of nine rats at seven weeks). Urinary pH was varied by feeding ammonium chloride or sodium carbonate in the drinking water. The rats excreting acid urine (pH 5.7) showed more extensive changes than did either of the other two groups (pH 6.7 and 7.9), not only in terms of primary papillary concretions but also as to dislodged fragments at lower levels of the urinary tract and hydronephrosis.

Supplemental vitamin B_6 completely prevented the oxalate deposits even when as much as 10 per cent of supplemental glycine was fed.

Studies of the effects of dietary levels of casein, glycine, and desoxypyridoxine (a B_6 antagonist) on endogenous oxalate synthesis in vitamin B_6-deficient rats were reported by Gershoff and Faragalla (*J. Biol. Chem.* **234,** *2391 (1959)*). On the basis of 48 hour urine collections, vitamin B_6 deficiency in rats increased oxalate excretion about two- to threefold. Desoxypyridoxine further increased the excretion.

Addition of 3 per cent of glycine, although not changing oxalate excretion much when 40 per cent of casein was fed, did increase urinary oxalate when 15 per cent of casein was fed. The highest oxalate excretion occurred when the 40 per cent casein diet was fed with desoxypyridoxine and 3 per cent of glycine, the urine containing about ten times the oxalate present when 15 or 40 per cent casein diets containing vitamin B_6 were fed. The feeding of isonicotinic acid hydrazide, a compound used in the treatment of tuberculosis and known to increase the vitamin B_6 requirement, increased oxalate excretion fourfold when vitamin B_6 was omitted from the diet.

The independent studies of Calhoun, Jennings, and Bradley (*J. Nutrition* **67,** *237 (1959)*) also demonstrated the occurrence of oxalate calculi in pyridoxine-deficient rats. Furthermore, these authors indicated that an amount of vitamin B_6 greater than that adequate for maximum growth was required to completely prevent oxalate excretion. They also demonstrated that inclusion of phthalylsulfathiazole in the vitamin B_6-deficient diet induced a hematuria accompanying the oxalate effects. Both groups of authors suggested the possible use of urinary oxalate as a criterion of vitamin B_6 adequacy of the diet.

These discoveries provide a new approach to the study of the human diseases in which oxalate plays a role, especially those of renal stone formation and oxalosis. These problems were reviewed in the paper by Gershoff *et al.* (*Am. J. Med.* **27,** *72 (1959)*). Studies of mental patients (Gershoff, A. L. Mayer, and L. L. Kulczycki, *Am. J. Clin. Nutrition* **7,** *76 (1959)*) indicated that supplemental pyridoxine reduced significantly the urinary oxalic acid excretion even though the diet of the patients presumably contained adequate amounts of vitamin B_6. Clearly more research is needed on the relationship of vitamin B_6 to the oxalate problem in man.

A more recent report (Gershoff and Andrus, *Fed. Proc.* **19,** *253 (1960)*) suggests that the vitamin B_6 effects on oxalate are markedly affected by the amount of dietary magnesium. Rats were fed purified diets containing 20, 40, or 400 mg. per cent of magnesium and 0, 0.1, or 0.4 mg. per cent of vitamin B_6. Oxalate calculi developed only in those animals without vitamin B_6 and in those fed the two lower levels of magnesium. The high dietary magnesium protected against oxalate deposition in the vitamin B_6-deficient animals. This investigation was probably stimulated by the report of G. Hammarsten in 1937 (cited by Andrus *et al.*, *Lab. Invest.* **9,** *7 (1960)*) which describes calcium oxalate calculi in rats fed natural diets considered to be low in magnesium (44 mg. per 500 g. of diet). The diets were also probably suboptimal with regard to vitamin B_6 content.

These interesting papers establish the importance of endogenous oxalate production in the development of oxalate calculi and oxalosis and the important role of vitamin B_6 and magnesium in preventing production of oxalate. Oxalic acid is apparently metabolically inert and is normally not produced to any great extent. However, if a precursor such as glyoxylic acid (a metabolite of glycine) is formed in large amounts and is not readily oxidized to CO_2 and H_2O, oxalic acid is formed (H. I. Nakada and S. Weinhouse, *Arch. Biochem. Biophys.* **42,** *257 (1953)*).

In view of the established biochemical relationships of vitamin B_6 coenzymes with ions such as Mg^{++} in the metabolism of amino acids and the relationship of glycine in the present studies and in those on human patients with hyperoxaluria (*Nutrition Reviews* **18,** *159 (1960)*), it is tempting to speculate on the mechanisms involved in the oxalate production. The real answers will become apparent only through further investigations.

BETA-GLUCURONIDASE AND ATHEROSCLEROSIS

A relationship was noted between the level of beta-glucuronidase and the production of atherosclerosis in chickens and possibly in man.

The metabolic disorders responsible for atherosclerosis have not been delineated although abnormalities in lipid metabolism appear to play a prominent role in its development. Thyroid and estrogenic hormones lower elevated plasma cholesterol levels in the blood. The same hormones have been found to increase the beta-glucuronidase activity in the blood.

Recently, S. Kayahan (Lancet **II,** *667 (1960)*) noted that the serum beta-glucuronidase activity was low in a group of animals suitable for producing experimental atherosclerosis and high in a group of animals unsuitable for this purpose. Kayahan investigated the relation of beta-glucuronidase to the production of atherosclerosis in chickens.

He had three groups of ten chickens; the first group was fed commercial chicken mash, the second and third groups were given the mash plus 2 per cent cholesterol and 5 per cent cottonseed oil. In addition, the third group received an injection of 500 units of beta-glucuronidase per day. These studies were carried out for ten weeks.

A fourth group of chickens was given the diet that would induce atherosclerosis and, in addition, received 800 units of beta-glucuronidase per day for 14 weeks. The animals receiving the diet without the cholesterol had no atherosclerosis, while those receiving the cholesterol had atherosclerosis graded at 0.7 to 2.8 degrees. The two groups of animals receiving cholesterol and the beta-glucuronidase averaged 0.1–0.5 degree atherosclerosis. The average serum total lipid level

expressed as mg. per 100 ml. was 306 for the control group and 4854 for the animals on the atherosclerotogenic diet. The lipid levels for the third and fourth groups of chickens receiving the beta-glucuronidase were 824 and 1862 mg. per 100 ml. respectively. The serum cholesterol increased about tenfold in the animals on the cholesterol-containing diet and was a half to three quarters of this level in those receiving the beta-glucuronidase.

Thus, it appears that the animals receiving the beta-glucuronidase had lower levels of both cholesterol and total serum lipids. The serum beta-glucuronidase of the chickens fell when they were developing atherosclerosis. The chickens receiving the injections had levels approximately 1.2 times that of the control animals. It would be interesting if these investigators had injected inactivated beta-glucuronidase to see whether or not the protein that the chickens were receiving along with the enzyme activity might have some influence on the blood lipid levels.

In the study of beta-glucuronidase activity of the aortic intima in 20 individuals who did not have atherosclerosis and 20 patients with atherosclerosis it was found that the beta-glucuronidase activity in the aortic intima of patients with atherosclerosis varied from 0 to 5.7 units per gram of tissue with an average of 2.3, while those of the non-atherosclerotic individuals varied from 12.9 to 16.6 with an average of 14.2. The serum beta-glucuronidase activity in the atherosclerotic patients was also below the non-atherosclerotic patients, the two averages being 6.20 and 9.8. Ten individuals who had atherosclerosis were given beta-glucuronidase intramuscularly at the rate of 25,000 units per day for 15 days then given weekly injections for two more weeks.

Comparing the pretreatment level of total lipids and total cholesterol in ten individuals, this investigator finds significant decreases in both of these lipids in all ten individuals who received the beta-glucuronidase. Also, all ten patients improved clinically. The question arises whether or not there is a definite cause- and effect-relationship between the beta-glucuronidase and lipid metabolism or whether the changes noted were the result of some secondary reaction as administration of a foreign protein to these individuals.

RELATION OF VITAMIN A TO MUCOPOLYSACCHARIDE FORMATION

By using preparations of colon from rats deficient in vitamin A, it was possible to demonstrate that the rate of mucopolysaccharide formation was inhibited by a lack of this vitamin and restored by replacement of it.

Chemically collagenous tissues comprise the largest substance of the body. Intimately associated with such tissues are the mucopolysaccharides which form the ground substance (*Nutrition Reviews* **11,** *82 (1953)*).

Deficiency of vitamin A results in disorders of many structures, chiefly epithelial tissues; but in the growing animal collagenous tissues also are particularly affected. Conversely, hypervitaminosis A affects the same tissues adversely (*Nutrition Reviews* **12,** *268 (1954)*). The actual mechanism of vitamin A action is ill-defined, but certainly it relates to metabolism of mucopolysaccharides.

Some clarification of this problem is provided in a report by G. Wolf and P. T. Varandani (*Biochim. Biophys. Acta* **43,** *501 (1960)*). The authors were prompted to study the effect of vitamin A on mucopolysaccharide formation as a result of reports indicating that excesses of vitamin A added to tissue cultures of bone cells, inhibited cartilage formation, and resulted in transfer of sulphate groups from chondroitin to soluble compounds in the surrounding medium

(H. B. Fell, E. Mellanby and S. R. Pelc, *J. Physiol.* (*London*) **134,** *179* (*1956*); **119,** *470* (*1953*)). Excessive amounts of vitamin A in cultures of embryonic chick ectoderm caused formation of mucous-secreting ectoderm and inhibited keratinization. Another group, studying connective tissue, found that lack of vitamin A promoted sulphate uptake by mucopolysaccharides and the addition of this vitamin decreased sulphate uptake (D. L. Frape *et al*, *J. Nutrition* **68,** *189* (*1959*)).

Wolf and Varandani employed preparations of rat colons, both intact segments and homogenates, to study the rate of mucopolysaccharide formation. Tissues were taken from normal animals and from animals deficient in vitamin A. These were incubated with solutions of S^{35} sulphate in Krebs-Ringer phosphate buffer. The mucopolysaccharides formed by each preparation were separated and identified by chromatographic, electrophoretic and immunologic techniques. The quantity of mucopolysaccharides formed by each preparation could then be estimated by the amount of radioactivity of the S^{35} contained therein.

It was found that preparations made from normal animals incorporated S^{35} more rapidly than did those from vitamin A-deficient animals. Furthermore, addition of vitamin A as the alcohol, the acid or the aldehyde, to the test system resulted in a more rapid rate of production of mucopolysaccharides.

Addition of 10 μg. of vitamin A increased the rate of polysaccharide formation of vitamin A-deficient gut to normal. Graded amounts of vitamin A were added to different preparations and it was found that between 10 and 15 μg. per gram of protein in the test system was optimal.

The specificity of this response was tested by adding vitamin D, vitamin K_1, or vitamin E, respectively, to separate preparations. Neither vitamins D nor K_1 had any effect, but vitamin E had a slight effect in augmenting the rate of mucopolysaccharide formation. The addition of both vitamins A and E to a preparation had no additive effect.

Addition of lipoxidase to a preparation (to destroy vitamin A) inhibited mucopolysaccharide formation markedly; addition of large quantities of vitamin A restored this function.

The authors speculated upon the biochemical steps which might be involved in the relationship between vitamin A and mucopolysaccharide synthesis. Although they admitted that information was inadequate to make a positive statement, they postulated that the most likely site of action was on polymerization of uridine diphosphate derivatives or on the activation of transfer of sulphate to this polymer.

This report provides a realistic explanation, based upon sound observation, for the clinical effects that have been observed in vitamin A deficiency. A technique such as this should enable investigators to explore other functions of this essential vitamin.

DIETARY MAGNESIUM AND EXPERIMENTAL ATHEROSCLEROSIS IN RATS

High saturated fat diets containing cholesterol and cholic acid and low magnesium produce high serum cholesterol and vascular sudanophilia, which can be partially prevented or reversed by increased magnesium or thyroxine.

Dietary factors influencing the deposit of lipids in the intima of the arteries of experimental animals have been studied in great numbers in recent years. These have generally been macronutrients such as fat and protein, and micronutrients such as vitamins or drugs with a wide range of activities (*Nutrition Reviews* **17,** *62* (*1959*)).

Hormonal administration has also been found to both combat and enhance lipid deposition.

D. M. Hegsted and his collaborators (*J. Exp. Med.* **106,** *757 (1957)*; **106,** *767 (1957)*) starting with the premise that excess thyroxine increases and thiouracil decreases the extent of atherogenesis in experimental animals on an atherogenic diet, noted the similarity of symptoms of hyperthyroidism and magnesium deficiency. This idea led them to investigate the effect of dietary magnesium on atherogenesis in rats on diets containing cholesterol and cholic acid with hydrogenated cottonseed oil as the sole source of fat; they found that such diets increased the magnesium requirement some eight- to 16-fold. Moreover, it was of considerable interest that increased dietary magnesium appeared to produce lower aorta and heart valve sudanophilia, although it actually brought about increases in serum cholesterol levels. Since supplementary thyroxine resulted in decreases of both serum cholesterol and intimal sudanophilia, it appeared first that thyroxine and magnesium do not have a common site of action, and second, that the magnesium effect represents the first clear-cut dissociation of serum cholesterol levels and intimal sudanophilia.

In a continuation of these experiments, (E. E. Hellerstein, M. Nakamura, Hegsted and J. J. Vitale, *J. Nutrition* **71,** *339 (1960)*; **71,** *347 (1960)*) the effect on serum cholesterol and vascular sudanophilia of different types of fat and varying levels of dietary magnesium was studied.

Weanling rats were fed diets varying in magnesium content from 24 to 192 mg. per 100 g. diet and containing, as the source of fat, corn oil or hydrogenated cottonseed oil at 5 or 20 per cent levels. Cholesterol (1 per cent) and cholic acid (0.3 per cent) were included in the diets of half the animals of each group to increase blood cholesterol levels. After 28 days on these diets, weight gain of all animals appeared to be independent of the dietary fat or magnesium alterations, but was decreased by the cholesterol-cholic acid supplement. As had been noted in the previous studies in the animals fed hydrogenated cottonseed oil, addition of the cholesterol and cholic acid resulted in decreased serum magnesium (with the lowest dietary levels) and greatly increased serum cholesterol. Increasing the dietary magnesium resulted in an increased serum level of this element and a small decrease in serum cholesterol in the animals not fed cholesterol (105 to 85 mg. per 100 ml. for the 5 per cent HCO group). The cholesterol-fed animals, however, on the corn oil-containing diets, had considerably lower serum cholesterol values than those on hydrogenated cottonseed oil (means of 326 and 860 mg. per 100 ml. respectively) but neither was changed by alteration of the dietary magnesium. In a separate experiment, when the serum cholesterol of 20 per cent corn oil-fed animals was raised by increasing the supplement to 3 per cent cholesterol and 1 per cent cholic acid, it was found that increasing the dietary magnesium brought about a slight decrease in serum cholesterol (from 440 to 385 mg. per cent).

A different picture was seen when the left ventricular valves and aortas of the animals were graded as to the extent of sudanophilia. No deposition was found in arteries of animals without the cholesterol supplement. With the supplement all animals showed considerable sudanophilia but only in those on the hydrogenated cottonseed oil-containing diet were the scores lowered by increasing the dietary magnesium. When the serum cholesterol of the corn oil-fed animals was raised by tripling the cholesterol-cholic acid supplement, increasing the dietary magnesium from 24 to 192 mg. per 100 g. then lowered the heart score. The authors concluded from these results that only when the serum cholesterol was elevated above about 400 mg. per 100 ml. did increased magnesium have an effect. However, this study emphasized the fact that the etiology of atherosclerosis can be considered only in

the light of the relationship of a great variety of factors, none of which is entirely independent.

These experiments were carried out for only 25 days; it seemed possible that more definitive results might be obtained over a longer period, and that it might be possible to detect regression of lipid deposits. Experiments were designed to check these points. The diets used were similar to those of the previous ones with the fat (hydrogenated vegetable oil) at 20 per cent and magnesium added at 48 mg. per 100 g.

After 25 days on this diet, animals had a serum cholesterol level of 672 mg./100 ml. and a heart score (a measure of sudanophilia of the left ventricular valves and thoracic aorta) of 4.8. Increasing the magnesium content of the diet to 192 mg. per 100 g. resulted in a serum cholesterol level of 851 mg. per 100 ml. but a decreased heart score (3.0). Thyroxine (1 or 2 mg. per 100 g. diet) resulted in decreases in both serum cholesterol (290 to 390) and heart scores (1.0 to 1.3).

When groups of rats were continued on the low magnesium diet for 60 days, the heart scores rose to a mean of 6.2, whereas if the magnesium supplement was increased to 192 mg., the heart score remained at 4.8. For one group of rats on the high magnesium diet, a heart score of 3 at 25 days fell to 1.6 at 60 days. Thyroxine also appeared to bring about regression of the sudanophilia from 4.8 (assumed from rats on the same diet) to 1.5 or 2 even on the low dietary magnesium. For some animals kept for six to 12 months on the low magnesium diet, the sudanophilia had apparently led to intimal plaque formation.

The conclusions drawn from these experiments are: 1) a high serum cholesterol, produced in rats by a cholesterol-cholic acid, high saturated fat diet, appears to increase the need for dietary magnesium; 2) if this need is not met, a high vascular sudanophilia results; 3) an increase in dietary magnesium does not lower the serum cholesterol, but both deters the development of sudanophilia (provided the serum cholesterol level is above about 400 mg. per 100 ml.) and brings about a regression of the lipid deposits already formed; 4) thyroxine efficiently prevents high serum cholesterol and aortic sudanophilia and promotes regression of both; 5) the rat is not entirely similar to the human being, either in nature of the disease or in its response to dietary and other treatment, but it still may be used as a convenient experimental model.

NOTES

Life Span and Pathology of Rats Maintained at 6°C and 30°C

Rats maintained at an environmental temperature of 1° to 3°C for ten to 18 months have increased deposition of fat in the coronary arteries and testicular hypoplasia with complete disappearance of spermatogenesis.

Recently, O. Heroux and J. S. Campbell (*Lab. Invest.* **9,** *305 (1960)*) studied the pathology and life span of 6°C- and 30°C-acclimated rats. They used 23 male Sprague-Dawley rats which were 50 days old and weighed about 200 g. The animals were divided into two groups: 13 maintained at 6°C and ten maintained at 30°C. Both groups were fed a commercial rat chow and water ad libitum.

These investigators found that the average life of the cold-acclimated rat was 560 ± 41 days while that of the animal living at 30°C was 708 ± 62 days. This latter figure agrees with the 742-day average life span of this breed of rat maintained at room temperature.

Upon microscopic examination of the tissues of the animal they concluded that the frequency of testicular hypoplasia, glomerulonephritis, myocardial fibrosis, and periarteritis nodosa in the rats maintained at 6°C was much greater than that of rats living their full life at 30°C. However, they noted that only a few of the cold-exposed rats developed pulmonary inflammation and none developed neoplasia.

These investigators suggest that perhaps environmental stress from cold may modify time of appearance and incidence of naturally occurring diseases without reducing the life span proportionately. They consider that the reduction in the average life span of the cold-exposed rats was "of meager statistical significance."

Radioactive Cholesterol Labelling of Serum Lipoproteins

Modern investigations of cholesterol metabolism demand elegant techniques capable of providing precise measurements. One problem in the use of radioactive cholesterol has been the rapid dilution of this material following its absorption from the alimentary tract. Parenteral administration of such labelled cholesterol has been difficult due to faulty dispersion of the material. A report by A. F. Whereat and E. Staple offers a reasonable solution for this problem (*Arch. Biochem. Biophys.* **90,** *224 (1960)*). They dissolved radioactive cholesterol in benzine, evaporated it to dryness, and dispersed it in Tween 20 dissolved in methanol. After evaporation of the methanol, a dilute solution of sodium chloride was added. Small quantities of this saline dispersion of radioactive cholesterol then were incubated with serum or plasma taken from human beings or rats. After varying periods of time these sera or plasma samples were studied by zone electrophoresis on starch, by ultracentrifugation, and by other methods to determine whether any of the proteins had been denatured and to measure the location of the radioactive tag. It was found that in human serum the radioactive level migrated with the alpha and beta globulins; in the rat the label migrated with a broad albumin peak as reported previously (A. G. Gornall, C. J. Bardawill and M. M. David, *J. Biol. Chemistry* **177,** *751 (1949)*).

Ultracentrifugation disclosed that the labelled cholesterol had united with the two lipoprotein classes having specific gravities greater than 1.063 and between 1.063 and 1.21. Analytical ultracentrifugation showed that the lipoproteins containing the labelled compounds compared identically with those of unlabelled lipoproteins from the same source.

By this technique it should be possible to prepare isotopically labelled cholesterol lipoprotein complexes which behave in a natural fashion and can be administered parenterally. This should allow more accurate evaluation of the metabolism of cholesterol and lipoprotein.

Citral, Vitamin A, and Glaucoma

In 1956 E. H. Leach and J. P. F. Lloyd reported that a factor present in the peel of citrus fruit might cause a form of simple glaucoma in monkeys and rabbits (*Tr. Ophth. Soc. U. Kingdom* **76,** *453 (1956)*) and (*Proc. Nutrition Soc. England and Scotland* **15,** *15 (1956)*). This compound known as "citral" is an unsaturated aldehyde. It is likely to be consumed in the form of marmalades, candied fruit peels, and in essences derived from citrus fruit peel.

According to Leach and Lloyd subcutaneous administration of 10 μg. of citral to rabbits would raise the intraocular pressure and larger doses would cause changes suggestive of vitamin A deficiency. Furthermore, they stated that this toxicity could be prevented or reversed by the administration of vitamin A or by giving sulfhydryl compounds such as cysteine, or British Anti-Lewisite (BAL). They suggested that this aldehyde exerted its harmful effect by blocking the use of vitamin A in the endothelial cells of the trabeculae resulting in disruption of endothelial metabolism with subsequent increase in the resistance to outflow from the anterior chamber. These findings have been quoted widely in journals dealing with ocular diseases and in reviews of nutrition (E. Linnér, *Brit. J. Ophth.* **52,** *38 (1958)*); (T. Moore, *Vitamin A, London, Elsevier (1957)*); (*Ann. Rev. of Biochem.*, **26,** *280 (1957)*).

F. C. Rodger and H. Saiduzzafar (*Am. J. Ophth.* **50,** *309 (1960)*) attempted to repeat these studies in rabbits, but failed. The authors employed large numbers of animals and a number of techniques with varying doses of citral, yet their results were the same. They even induced a deficiency of vitamin A in some rabbits and gave a wide range of dosage of citral to the animals, but still the ocular pressures remained normal. They concluded that neither citral nor vitamin A have any significant effect on intraocular pressure or on the factors which regulate it in animals.

These results seem conclusive and should end the discussion of citral in relation to glaucoma.

Recent Books

Control of Malnutrition in Man. Companion volume to *Control of Communicable Diseases in Man.* 9th edition. Published by the American Public Health Association, Inc., 1790 Broadway, New York 19, New York. Pp. 132.

La Alimentacion y El Estado de Nutricion en el Peru. Carlos Collazos Chiriboga *et al.* Ministerio de Salud Publica Y Asistencia Social, Servico Cooperativo Interamericano de Salud Publica, Instituto de Nutricion, Lima, Peru. Pp. 343.

The Effect of High Temperature Storage on the Acceptability, Digestibility, and Composition of the U.S. Army Ration, Individual, Combat. Report 228. October 1958. U.S. Army Medical Research and Nutrition Laboratory. Fitzsimons Army Hospital. Denver 8, Colorado.

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